Antibacterial activity of *Morinda citrifolia* Linneo seeds against Methicillin-Resistant *Staphylococcus* spp

Natividad Giovana De La Cruz-Sánchez, Abraham Gómez-Rivera, Patricia Alvarez-Fitz, Elsa Ventura-Zapata, Ma Dolores Pérez-García, Margarita Avilés-Flores, Ana Silvia Gutiérrez-Román, Manasés González-Cortazar

**Abstract**

In traditional medicine, *Morinda citrifolia* (Noni) is used to treat various ailments, including skin and respiratory-tract infections. In this work, a bio-directed study (seed extracts) with five bacteria was carried out against four clinical isolates of Methicillin-Resistant *Staphylococcus* (MRS) and *Staphylococcus aureus* ATCC 29213 strain to find molecules capable of inhibiting them. Three organic extracts were obtained by maceration of the noni seeds with ascending polarity solvents (n-hexane, dichloromethane and methanol) that were evaluated as antibacterial in the model of bioautography and broth microdilution techniques. The results showed that the methanolic extract was the most active against all bacteria (MIC = 16 μg/mL). The chromatographic fractionation performed on this extract allowed obtaining six fractions (EMF1-EMF6), of which F1, F2 and F5 exhibited activity against some of the bacteria. EMF1 fraction reached an MIC of 25 μg/mL against *S. haemolyticus* twice as much as the positive control, in which the chemical content is mainly composed of a mixture of γ-butyrolactones (1–2) and esterified fatty acids (3–9); chemical characterization of the nine compounds was carried out based on gas chromatography coupled to masses. EMF2 fraction, presented an MIC of 200 μg/mL against *S. aureus* 0198 and *S. haemolyticus* 562B, where a coumarin known as scopoletin (10) was isolated and active against *S. aureus* 0198 (MIC = 100 μg/mL). EMF5 fraction demonstrated an MIC of 200 μg/mL against *S. aureus* 0198, *S. haemolyticus* 562B and *S. epidermidis* 1042, in which a neolignan known as americanin A (11) was identified, showing activity against *S. haemolyticus* 562B and *S. epidermidis* 1042 (MIC = 100 μg/mL). The chemical characterization of isolated compounds 10 and 11 was performed by the analysis of 1H and 13C NMR. Therefore, the methanolic extract, identified and isolated compounds showed important antibacterial activity against the MRS, validating its use in traditional medicine.

**Keywords:**
- Morinda citrifolia
- Antibacterial activity
- MRS
- Fatty acids
- γ-butyrolactones
- Scopoletin
- Americanin A

**Abbreviations:**
- MRS, Methicillin-Resistant *Staphylococcus*
- MIC, Minimal Inhibitory Concentration
- MRSAs, Methicillin-Resistant *Staphylococcus aureus*
- EH, n-hexane extract
- ED, dichloromethane extract
- EM, methanol extract
- TLC, Thin-Layer Chromatography
- CFU, Colony-Forming Units

**Corresponding author.**

E-mail address: gmanases2000@gmail.com (M. González-Cortazar).

https://doi.org/10.1016/j.micpath.2019.01.030

Received 17 October 2018; Received in revised form 11 January 2019; Accepted 18 January 2019

Available online 23 January 2019

© 2019 Elsevier Ltd. All rights reserved.
such as teicoplanin (glycopeptide) and linezolid (oxazolidinone) are used; however, they have limited availability and adverse effects, respectively [4].

Other Methicillin-resistant bacteria of clinical importance and related to nosocomial infections include S. haemolyticus and S. epidermidis [5–8]. Thus, other treatment options are required that can inhibit the growth of these bacteria.

*Morinda citrifolia* L., is a small tree or bush, belongs to the Rubiaceae family, is one of the 80 species of *Morinda* genus [9,10], is known as “noni” in the traditional medicine fruits, leaves, roots and seeds are employed for different ailments such as fevers, infections, diarrheaa, constipation, asthma, insect bites, animal bites, to the relieve nausea caused by chemotherapy, and ulcers [10–12]. In the Mexican state of Veracruz, the fruits are used for the treatment of diabetes, tuberculosis, ulcers and infections [13]. Pharmacological studies have shown antioxidant, antitumor, cardiovascular and antibacterial activities in noni roots, leaves and seeds [14,15]. Scientific reports have shown that extracts hexanic, chloroformic, ethyl acetate, alcoholic and acetonitrile mobile phase in gradient system (10 mL samples), to obtain 46 fractions that were grouped according to the similarity of the compounds in 16 (EMC2F1-EMC2F16) according to similitude of compounds. In the EMC2F9 fraction (20 mg) was identified the compound (11).

The structure of compounds 10 and 11 was identified by analysis of the 1H and 13C NMR spectra acquired in Varian Inova 400 at 400 MHz, employing as solvent CDCl3 and NMR (1H and 13C) acquired in a Bruker advance III HD-600 at 600 MHz equipment using CD3OD solvent respectively. Chemical shifts are reported in ppm relative to TMS.

2.5. Antibacterial activity

2.5.1. Bacterial clinical isolates

The five bacterial strains used were the following: *Staphylococcus aureus* ATCC 29213 (sensitive strain) and four clinical methicillin-resistant isolates including *Staphylococcus aureus* 0198, *Staphylococcus haemolyticus* 562B, *Staphylococcus epidermidis* 1042 and *Staphylococcus haemolyticus* 731B, isolates at the Hospital General de Acapulco, Mexico and which were provided by the Universidad Autónoma de Guerrero (UAGO). The strains were maintained on Trypticase Soy Agar (Merck) at 37 °C, 24 h.

2.5.2. Bioautography assay

Bioautography method was carried out according to that described by Navarro et al., 1998 [22] with strains of *Staphylococcus aureus* (ATCC 29213). The method consists of developing normal-phase silica-gel TLC using different solvent systems for each extract as mobile phase: EH (hexane-dichloromethane 90:10 v/v), ED and EM (dichloromethane-methanol 95:5 and 90:10 v/v respectively). At the end of the elution, the plates were impregnated with a suspension of *S. aureus* (1 × 10⁸ CFU/mL) during 37 °C for 24 h, later the TLC was revealed with an aqueous solution of 2,3,5-triphenyltetrazolium chloride and allowed to stand for 15 min at 37 °C. Growth inhibition is observed as a white zone on a pink background indicating bacterial growth.

2.5.3. Minimal inhibitory concentration (MIC)

The MIC of the extracts, fractions and compounds were determinate by broth microdilution technique according to the CLSI (Clinical and Laboratory Standards Institute, 2013) [23], with minimal modifications [24]. For the assays, 3–4 colonies from each of the strains were taken to evaluate and be reseeded in Müeller-Hinton broth (Merck), these were incubated at 37 °C for 3 h. After this, were compared and adjusted the 0.5 scale of turbidity with McFarland standard colony-forming units per milliliter (1 × 10⁶ CFU/mL) and diluted with sterile distilled water, corresponding to a final concentration of 1 × 10⁸ (CFU/mL).

The seeds of *Morinda citrifolia* fruits were obtained and collected at the Centro de Investigación Biomédica del Sur (CIBIS) greenhouse’s, in Xochitepec, Morelos State, Mexico, in April 2016. Safekeeping and taxonomic identification were carried out at the Jardín Etnobotánico y Museo de Medicina Tradicional y Herbario del Instituto Nacional de Antropología e Historia (INAH) with registration under the following voucher: 2057.

2.2. Extraction and isolation

The seeds were separated from the fruits, washed with water and dried using oven-dried at 50 °C for 24 h (687.3 g). Then the dried seeds were pulverized using Pulvex MPP300 mill. A total of 500 g of seeds was macerated with n-hexane (EH), dichloromethane (ED) and methanol (EM) (for 2 days). Liquid extracts were filtered using Whatman No. 4 filter paper and concentrated under reduced pressure in a rotatory evaporator (Heidolph G3) until the extracts were completely dry obtaining the following yields: EH 5.79%, ED 1.48% and EM 2.95%. The most active extract (EM, 20.3 g) was subjected to chromatographic fractionation on a silica gel normal phase column (25 g, 46 × 2.5 cm) with dichloromethane/methanol gradient system with an increase in polarity of 5% to render 37 samples that were grouped in nine fractions (EMC1F1-EMC1F9). In the EMC1F4 fraction (10.9 mg) compound 10 was identified.

EMF5 (1.4 g) was subjected to chromatographic fractionation in a reverse-phase column (LC-18 Packing, 10 g, SUPELCO) with a water/acetonitrile mobile phase in gradient system (10 mL samples), to obtain 46 fractions that were grouped according to the similarity of the compounds in 16 (EMC2F1-EMC2F16) according to similitude of compounds. In the EMC2F9 fraction (20 mg) was identified the compound (11).

The chemical composition of EMF1 (113.2 mg) was analyzed on a Gas Chromatograph-Mass Spectrometry (GC-MS) equipped with a quadrupole mass detector in electron impact mode at 70 eV. Volatile compounds were separated onto a HP 5MS capillary column (25 m long, 0.2 mm i.d., with 0.3-µm film thickness). Oven temperature was set at 40 °C for 2 min, then programmed from 40 to 260 °C at 10 °C/min and maintained for 20 min at 260 °C. Mass detector conditions were as follows: interphase temperature 200 °C and mass acquisition range, 20–550. Carrier gas was helium at a flow rate of 1 mL/min. Identification of the compounds was performed with the comparison of mass spectra with those of the National Institute of Standards and Technology (NIST, 1.7 Library) and with data from the literature [21] which identified nine compounds (1–9).
Samples (extracts, fractions and compounds) were dissolved in 1 mL of a mixture (200 μL DMSO + 800 μL sterile water). The method was carried out on sterile 96-well microplates. 100 μL of each of the samples was added to the evaluated concentrations and 2 μL of the inoculums of each bacterium. The antibiotic utilized as positive control was ceftriaxone.

Serial dilutions were made to obtain concentrations of the extract at 2, 4, 6, 8 and 16 mg/mL, of the fractions at 25, 50, 100 and 200 μg/mL, of the compounds at 12.5, 25, 50 and 100 μg/mL and of the antibiotic at 2, 4, 6, 8 and 16 μg/mL adding 100 μL of Müeller Hinton broth to each one. The negative control was constituted by the mixture of Müeller Hinton broth, DMSO and inoculum. Evaluation was performed by duplicate.

Microplates were incubated at 37 °C for 24 h. After incubation, the MIC was determined according to the coloration generated by the reaction with 2,3,5-diphenyl-2H-tetrazolium bromide (30 μL, 0.05%, MTI, Merck) added to each well, in where from yellow to purple is considered cell viability and no is considered change as inhibition.

3. Results and discussion

3.1. Chemical profile of fraction EMF1 by GC-MS

The analysis of EMF1 by GC-MS allowed the identification of nine compounds. In Table 1, these are presented and listed in order of elution, highlighting the presence of γ-butyrolactones such as 5-butyloxolan-2-one (1, 54.4%, majority compound) and 5-hexyloxolan-2-one (2, 12.06%). Also a long-chain fatty aldehyde called pentadecanal (3), fatty acid esterified as methyl palmitate (4, 3.04%), methyl linoleate (5, 6.44%), methyl oleate (6, 2.85%) and diethylhexyl adipate (8, 3.0%) and finally, aliphatics such as (E)-octacos-2-ene (9, 8.87%). These structures are illustrated in Fig. S5 (Supplementary Materials). This is the first time, to our knowledge, that the presence of the compounds (1–9) is reported in a seed extract of *M. citrifolia*, as well as the antibacterial activity of the mixture against *M. ruscans*.

3.2. Characterization of scopoletin (10)

EMCF14 fraction in the HPLC chromatogram a peak was observed with retention time of 10.7 min (Fig. 1a). The UV spectrum showed five absorption bands at $\lambda_{max}$ 208, 226, 297 and 343 nm (Fig. 1b), characteristic of coumarins such as scopoletin [25]. It was observed as yellow amorphous crystals, soluble in dichloromethane and in TLC with those previously described [29], allowed us to determine that this compound corresponds to (E)-3-[(3,4-dihydroxyphenyl)-3-(hydroxy)-prop-2-enyl] (10, Fig. 1) which is a neolignan called americanin A. This compound has been identified in this specie in leaves and fruits [27,28]. americanin A was isolated for the first time in seeds of *Phytolacca americana* [29].

3.3. Characterisation of neolignan (americanin A, 11)

In EMCF19 fraction, a yellow-brown precipitate soluble in methanol was obtained. In the HPLC chromatogram a peak was observed with a retention time of 10.08 min (Fig. 5a). The UV spectrum showed three absorption bands at $\lambda_{max}$ 208, 226 and 335 nm (Fig. 5b) which are characteristic of lignanes [14] and in a TLC reverse phase with chromogenic developers (NP-PEG) a dark coloration was observed in UV light (Fig. 3c).

The analysis of the spectroscopic data (Table 3) of $^1$H and $^13$C NMR spectra (Figs. S3 and S4, Supplementary Materials) and its comparison with those previously described [29], allowed us to determine that this compound corresponds to (E)-3-[2-(3,4-dihydroxyphenyl)-3-(hydroxymethyl)-2,3-dihydro-1,4-benzodioxin-6-yl]prop-2-enal (11, Fig. 4) which is a neolignan called americanin A. This compound has been identified in this specie in leaves and fruits [27,28]. americanin A was isolated for the first time in seeds of *Phytolacca americana* [29].

3.4. Antibacterial activity by bioautographic method

Antibacterial activity of *Morinda citrifolia* extracts by bioautography assay is shown in Fig. 5. All the extracts (EH, ED and EM) were active and to present a zone of inhibition in this biological model against *S. aureus* (ATCC 29213) strain. Hexane extract (EH) showed a zone of inhibition (Fig. 5a) where metabolites of low and medium polarity are located, while in the dichloromethane extract (ED) the zone of inhibition (Fig. 5b) was located with the compounds of low polarity and for methanolic extract (EM) those of high polarity (Fig. 5c). In the latter, scopoletin and americanin A were obtained.

3.5. Antibacterial activity

Antibacterial activity determined by MIC values of the extracts EH, ED and EM against four MRS strains and a sensitive one is presented in Table 4. EM was the most active inhibiting all the strains evaluated with an MIC of 16 mg/mL, followed by EH that also inhibited four of them (*S. aureus* 019B, *S. haemolyticus* 562B, *S. haemolyticus* 731B and *S. aureus* 0198) with an MIC of 16 mg/mL and finally ED, the least active, showing only inhibition against one strain (*S. epidemidis* 1042) with an MIC of 16 mg/mL. This is consistent with what has been reported: an ethanolic extract of *Morinda citrifolia* showed antibacterial activity against a strain of *S. aureus* with an MIC of 32 mg/mL [30].

Previous studies have determined that *M. citrifolia* seed extracts of higher polarity such as acetone, chloroform and methanol possess antibacterial activity against Gram-positive (*S. aureus*), Gram-negative (*Salmonella* spp, *Escherichia coli*, *Pseudomonas aeruginosa*, *Helicobacter pylori* and *Shigella* spp) [12]. The observed antimicrobial effect in *M. citrifolia* may be due to presence of phenolic compounds such as acubin, L-asperuloside, alizarin, scopoletin and other anthraquinones [31].

Table 1

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Molecular weight (a.m.u.)</th>
<th>Compound</th>
<th>% In the sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.30</td>
<td>142</td>
<td>5-Butyloxolan-2-one (1)</td>
<td>54.40</td>
</tr>
<tr>
<td>13.96</td>
<td>170</td>
<td>5-Hexyloxolan-2-one (2)</td>
<td>12.06</td>
</tr>
<tr>
<td>16.75</td>
<td>226</td>
<td>Pentadecanal (3)</td>
<td>2.19</td>
</tr>
<tr>
<td>18.96</td>
<td>270</td>
<td>Methyl hexadecanoate (Methyl palmitate, 4)</td>
<td>3.04</td>
</tr>
<tr>
<td>20.60</td>
<td>294</td>
<td>Methyl (9Z,12Z)-octadeca-9,12-dienoate (Methyl linoleate, 5)</td>
<td>6.44</td>
</tr>
<tr>
<td>20.65</td>
<td>296</td>
<td>Methyl (Z)-octadec-9-enoate (Methyl oleate, 6)</td>
<td>2.85</td>
</tr>
<tr>
<td>20.87</td>
<td>298</td>
<td>Methyl octadecanoate (Methyl stearate, 7)</td>
<td>1.22</td>
</tr>
<tr>
<td>23.42</td>
<td>370</td>
<td>Bis (2-ethylhexyl) hexadecanoate (Diethylhexyl adipate, 8)</td>
<td>3.00</td>
</tr>
<tr>
<td>33.12</td>
<td>392</td>
<td>(E)-Octacos-2-ene (9)</td>
<td>8.87</td>
</tr>
</tbody>
</table>
Therefore, the results obtained from the activity of *M. citrifolia* seed extracts against MRS can be an alternative for the treatment of diseases related to antibiotic resistant pathogens and the activity could be improved when looking for new formulations such as what was achieved when employing liposomes to encapsulate cinnamon oil and clove oil showing an excellent antibacterial effect on MRSA and MRSA biofilms [32,33].

### 3.6. Antibacterial activity of the fractions and compounds from EM

The *in vitro* antibacterial activities of the fractions (EMF1-EMF6) obtained from the methanolic extract of *M. citrifolia* (seed) are presented in Table 5. The fractions that exhibited inhibition in some of the microorganisms tested principally against the MRS strains were EMF1, EMF2 and EMF5, with an MIC of 25 at 200 mg/mL.

The EMF5 fraction was the most active because it demonstrated inhibition against *S. aureus* 0198, *S. haemolyticus* 562B and *S. epidermidis*...
1042 with an MIC of 200 μg/mL, respectively. EMF2 showed activity only against *S. aureus* 0198 and *S. haemolyticus* 731B with a MIC of 200 μg/mL, respectively. Finally, EMF1 only showed inhibition against *S. haemolyticus* 731B but with an MIC of 25 μg/mL, compared to the antibiotic (ceftazidime) which has an MIC of 64 mg/mL, this fraction is 2.56 times more potent as an antimicrobial, the later, could be due to the presence of γ-butyrolactones and esterified fatty acids (1–9) that were identified in EMF1.

![HPLC chromatogram](image1)

**Fig. 3.** a) HPLC chromatogram, b) UV spectrum and c) TLC reverse phase (60:40; waters/acetonitrile, UV light a 365 nm) of EMC2F9.

**Table 3**

NMR spectroscopic data (600 MHz, CD$_3$OD, δ ppm, J in Hz) of americanin A (11).

<table>
<thead>
<tr>
<th>Position</th>
<th>δ H (J in Hz)</th>
<th>δ C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>129.24</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6.87 (d, 1.56)</td>
<td>115.73</td>
</tr>
<tr>
<td>3</td>
<td>146.82</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>147.40</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>147.40</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>146.82</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>7.76 (dd, 2.3, 8.5)</td>
<td>155.40</td>
</tr>
<tr>
<td>8</td>
<td>6.63 (dd, 7.8, 15.8)</td>
<td>155.40</td>
</tr>
<tr>
<td>9</td>
<td>9.57 (d, 7.8)</td>
<td>196.20</td>
</tr>
</tbody>
</table>

![NMR spectrum](image2)

**Fig. 4.** (E)-3-[2-(3,4-dihydroxyphenyl)-3-(hydroxymethyl)-2,3-dihydro-1,4-benzodioxin-6-yl]prop-2-enal (11).

1042 with an MIC of 200 μg/mL, respectively. EMF2 showed activity only against *S. aureus* 0198 and *S. haemolyticus* 731B with a MIC of 200 μg/mL, respectively. Finally, EMF1 only showed inhibition against *S. haemolyticus* 731B but with an MIC of 25 μg/mL, compared to the antibiotic (ceftazidime) which has an MIC of 64 mg/mL, this fraction is 2.56 times more potent as an antimicrobial, the later, could be due to the presence of γ-butyrolactones and esterified fatty acids (1–9) that were identified in EMF1.
from EM of extracts that did show inhibition, could suggest that the presence of the secondary metabolites is required to be able to inhibit this microorganism.

Antimicrobial activity of compounds 11 and 12 obtained from the active fraction (EMF5) are listed in Table 5. Americanin A (11) showed antimicrobial activity against two MRS (*S. haemolyticus* 562B and *S. epidermidis* 1042) strains, both with an MIC of 100 µg/mL. Effect like the positive control (ceftazidime) with a MIC of 64 and 16 µg/mL, respectively. On the other hand, scopoletin (10) only presented inhibition against an MRS (*S. aureus* 0198) microorganism, with an MIC of 100 µg/mL.

With respect to compound (11), the activities that have been shown to this neolignan are as follows: antiproliferative and antitumor activity in colon cancer cells [34], antioxidant, larvicidic [27], antihapatotoxic [29] and anti-inflammatory in an arthritis model [35]. However, this is, to our knowledge, the first time that it is related with antibacterial activity against a resistant microorganism. Scopoletin (10) has been shown to have antibacterial activity against *E. coli* [36]. In addition to this compound (11), activity has also been reported as antithyroid, antioxidant, antihyperglycemic [37] and hepatoprotective [38].

Therefore, this is the first time that these compounds (10 and 11) have been evaluated against MRS strains of clinical isolates and that they have shown important activity against three of the five strains evaluated, such as *S. aureus, S. haemolyticus* and *S. epidermidis*, which are pathogens that have exhibited multiple resistance to antibiotics in nosocomial infections. In addition, specifically *S. aureus* is considered as the second infectious agent of clinical importance [6,39,40]. On the other hand, the activity that showed 10 and 11 could be higher if it is administered in a formulation that allows enhancing its effect, as reported for artemisinin, which was encapsulated in beta-cyclodextrin (β-CD) improving its solubility and a concentration of 20 mg/mL achieved an activity of 99.94% inhibition against MRSA after 4 days, the mechanism of antibacterial activity being an increase in membrane permeability and the respiratory metabolism of MRSA was inhibited via Embden-Meyerhof-Parnas pathway [41].

The clinical isolate of methicillin-resistant *Staphylococcus* (MRS) are the most common cause of infections among the global pathogenic bacteria, therefore, the search for novel anti-MRS agents is urgently needed. The molecular antibacterial mechanism of lignans and coumarins has been report; the lignans acts on the cellular membrane of MRSA repressing proteins of the ABC transport system [42] and inhibition of autolysis and biofilm formation in clinical MRS strains [43]. The coumarins are considered as potential antibacterial agents, and acts to inhibitor of the Penicillin Binding Proteins (PBP) from MRSA. Additionally, are excellent inhibitor of DNA gyrase (in *vivo*) by competing with ATP for binding to the β subunit of the enzyme. The antibacterial activity of coumarins it is due to its basic structure, benzopyrone, is structurally like benzyoprydione backbone basis structure for the quinolone's of antibacterial drugs [44–47]. The lignans and coumarins have a wide range of chemical structures, and they can serve as molecular templates for new antibacterial drugs.

## Table 4

**Antibacterial activity of *Morinda citrifolia* extracts (EH, ED and EM).**

<table>
<thead>
<tr>
<th>Extracts</th>
<th>MIC (mg/mL)</th>
<th><em>S. aureus</em> 0198</th>
<th><em>S. haemolyticus</em> 562B</th>
<th><em>S. epidermidis</em> 1042</th>
<th><em>S. haemolyticus</em> 731B</th>
<th><em>S. aureus</em> ATCC 29213</th>
</tr>
</thead>
<tbody>
<tr>
<td>EH</td>
<td>16</td>
<td>16</td>
<td>&gt; 16</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>ED</td>
<td>&gt; 16</td>
<td>&gt; 16</td>
<td>16</td>
<td>&gt; 16</td>
<td>&gt; 16</td>
<td>&gt; 16</td>
</tr>
<tr>
<td><strong>Ceftazidime</strong></td>
<td>32</td>
<td>64</td>
<td>16</td>
<td>64</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td><strong>Control</strong> (-)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

S: *Staphylococcus*; *Antibiotic control (µg/mL); ** Negative control (Mueller-Hinton + DMSO to 20% + inoculum strains).*

## Table 5

**Antibacterial activity of fractions (EMF1-EMF6) and compounds (10 and 11) from EM of *M. citrifolia*.**

<table>
<thead>
<tr>
<th>Fractions</th>
<th>MIC (µg/mL)</th>
<th><em>S. aureus</em> 0198</th>
<th><em>S. haemolyticus</em> 562B</th>
<th><em>S. epidermidis</em> 1042</th>
<th><em>S. haemolyticus</em> 731B</th>
<th><em>S. aureus</em> ATCC 29213</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMF1</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>25</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
</tr>
<tr>
<td>EMF2</td>
<td>200</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
</tr>
<tr>
<td>EMF3</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
</tr>
<tr>
<td>EMF4</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
</tr>
<tr>
<td>EMF5</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
</tr>
<tr>
<td>EMF6</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
</tr>
<tr>
<td>Scoptolin</td>
<td>100</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Americain A (11)</td>
<td>&gt; 100</td>
<td>100</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
</tr>
<tr>
<td><em>Ceftazidime</em></td>
<td>32</td>
<td>64</td>
<td>16</td>
<td>64</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td><strong>Control</strong> (-)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

S: *Staphylococcus*; *Antibiotic control (µg/mL); ** Negative control (Mueller-Hinton + DMSO (20%) + inoculum strains).*

The null activity against the sensitive strain (*S. aureus* ATCC 29213) presented by the six fractions in comparison with that of the methanol extract that did show inhibition, could suggest that the presence of all the secondary metabolites is required to be able to inhibit this microorganism.

4. Conclusions

This study demonstrates the antibacterial potential of the extract, fractions and compounds obtained from the seeds of *M. citrifolia* against four strains of MRS clinical isolates and a sensitive ATCC. Therefore, it concludes that the seeds of *M. citrifolia* can be used as a therapeutic alternative in the treatment of diseases related to MRS, reducing the time and severity of infections by these pathogens. These results even contribute to validating the traditional use of this species as an antibacterial and comprise the basis for upcoming studies against emerging pathogens with standardized extracts.

Acknowledgments

The Authors acknowledges the Instituto Mexicano del Seguro Social (register number project 2015-1702-9; research scholar...
References


